UvA-DARE (Digital Academic Repository)

Management of alloimmunized, refractory patients in need of platelet transfusions

Engelfriet, C.P.; Reesink, H.W.

DOI

[10.1046/j.1423-0410.1997.73301911.x](https://doi.org/10.1046/j.1423-0410.1997.73301911.x)

Publication date

1997

Published in

Vox Sanguinis

[Link to publication](#)

Citation for published version (APA):

Engelfriet, C. P., & Reesink, H. W. (1997). Management of alloimmunized, refractory patients in need of platelet transfusions. *Vox Sanguinis*, 73, 191-198. <https://doi.org/10.1046/j.1423-0410.1997.73301911.x>

General rights

It is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), other than for strictly personal, individual use, unless the work is under an open content license (like Creative Commons).

Disclaimer/Complaints regulations

If you believe that digital publication of certain material infringes any of your rights or (privacy) interests, please let the Library know, stating your reasons. In case of a legitimate complaint, the Library will make the material inaccessible and/or remove it from the website. Please Ask the Library: <https://uba.uva.nl/en/contact>, or a letter to: Library of the University of Amsterdam, Secretariat, Singel 425, 1012 WP Amsterdam, The Netherlands. You will be contacted as soon as possible.

Management of Alloimmunized, Refractory Patients in Need of Platelet Transfusions

C. P. Engelfriet
H. W. Reesink

There is now ample evidence that *primary* alloimmunization against HLA class I antigens is nearly always prevented if the number of leucocytes in all transfused cell concentrates is reduced to fewer than 5×10^6 . However, *secondary* immunization in patients who have been previously immunized by pregnancy or transfusion is not prevented. Neither can immunization against platelet-specific alloantigens be prevented by removing leucocytes from platelet concentrates, but platelet-specific antibodies seem to occur mainly in patients who develop HLA class I antibodies. Not all patients with HLA antibodies are refractory. Often the antibodies only react with a small percentage of panel lymphocytes and then most random donors are compatible. Furthermore, although there are individual differences, some HLA class I antigens are weakly expressed on platelets. Nevertheless, refractory alloimmunized patients in need of platelet transfusions will continue to occur. Opinions on the optimal management of such patients differ. Moreover, some simple and interesting new crossmatch techniques have recently been developed. For these reasons it seemed of interest to devote an International Forum to the subject.

There seem to be two main approaches to the management of refractory patients: (I) the selection of platelet concentrates based on the HLA class I phenotype and, or the specificity of the HLA (and platelet-specific) antibodies, and (II) the selection of platelet concentrates by crossmatching.

To obtain more information on the subject, the following questions were submitted to 15 experts in the field:

ad I: If an alloimmunized patient is in need of platelet transfusions:

(1) Do you determine the nature of the alloantibodies, i.e. HLA class I and/or platelet-specific?

(2) If the specificity of the HLA class I antibodies has been determined, do you merely select HLA-compatible donors or do you perform a crossmatch with HLA-compatible platelets as well, and if so which technique(s) is (are) used?

(3) If the specificity of the HLA class I antibodies is not or cannot be determined, do you select HLA class I antigen-compatible donors and if so, do you take splits of antigens into account?

(4) Do you have a panel of HLA-typed apheresis donors at your disposal?

(5) If platelet-specific antibodies are detected, do you have a panel of HPA-typed apheresis donors at your disposal?

ad II: If an alloimmunized patient is in need of platelet transfusions:

(1) Do you select compatible donors primarily by crossmatching with donor platelets?

(2) If so, which technique(s) is (are) used? Do you have experience with either of the two published new crossmatch techniques, i.e. the assay described by Ramos et al. [1] and that described by Ogden et al. [2]?

(3) Do you have at your disposal a panel of apheresis donors whose platelets are used in the crossmatch? If so, do you store these platelets and how?

Acid-treated platelets: Do you have experience with transfusions of acid-treated plate-

lets in patients with HLA class I antibodies [3]?

Contributions to the Forum were obtained from only 7 of the 15 invited experts. These contributions contain interesting information.

In all the centres from which information was obtained, the first step in providing platelets for alloimmunized, refractory patients, is to select HLA-matched platelets based on the phenotype of the patient. For this purpose, pools of HLA-typed apheresis donors, ranging from 2,000 to 22,000 donors, are available. The largest pool of 22,000 donors is divided over several cooperating blood banks in the Netherlands. In most centres, antigen splits are taken into account whenever possible. In some centres, efforts are made to define the specificity of the HLA antibodies and, if there is no donor with an acceptable match, antibody-compatible donors are selected if available. An investigation for platelet-specific antibodies is usually only done if HLA-matched, or compatible platelets do not induce a satisfactory increment, but in some centres it is done routinely. Platelet-specific antibodies were detected in 10% in one and in 20% of patients in another centre. No exact figures are provided by the other contributors. It is of interest that in 1 of 5 patients with HPA antibodies, no HLA class I antibodies were detectable [see Brand et al., below]. A panel of HPA-typed donors is available in all the centres. In some of the centres, if platelets selected by HLA phenotype did not induce a satisfactory increment, a crossmatch is done with platelets from donors with the best HLA match.

With the above approach, the percentage of successful transfusions ranges from 70 to 90% except in one centre in which only 50%

of fully-matched platelets produced a satisfactory result [see Murphy et al., below]. In one centre where 82% of transfusions of matched platelets have been successful, it is estimated that an additional crossmatch would increase this percentage to 90%. However, in the experience of the authors, about 8% of crossmatch-positive platelets produce a satisfactory increment [see Brand et al.]. In one centre it was found that, in order to find 1 or 2 compatible donors for highly immunized patients, 100 units of platelets had to be cross-matched. Posttransfusion increments of platelets selected in this way were successful in 80% of the cases. Interestingly the HLA type of some of the donors whose platelets gave a good increment was quite different from that of the patients [see Schiffer].

With regard to the techniques used for antibody detection, it is of interest that in one centre [see Contreras and Navarrete] an ELISA is used, in addition to the LCT, to detect HLA antibodies. In 7% of cases the ELISA is positive but the LCT negative due to the presence of noncomplement binding HLA class I antibodies. The use of DTT-treated serum is advocated because in many patients, particularly recipients of a bone marrow transplant, IgM lymphocyte autoantibodies are present which are not relevant for platelet transfusions.

No contributions were obtained from centres where platelets are primarily selected by crossmatching. We therefore have no new information on the relationship between primary crossmatch results and the success of platelet transfusions. The efficacy of the 'crossmatch policy' has been discussed in several papers. In a recent publication [4], it was reported that, whereas 62% of HLA-matched (A or B matches only [for an explanation of the various matches, see e.g. Brand et al., below], non-crossmatched platelets produced a 1-hour increment of >5,000, 72% of non-HLA-typed, crossmatch-negative platelets gave such results. For the crossmatch, the solid-phase Capture-P or Modified Capture-P kits were used. Furthermore, the percentage of successful transfusions of platelets with a B or C match increased from 38 to 67% if only crossmatch-negative platelets were used. None of the crossmatch-positive platelet transfusions produced a satisfactory increment. The results of one of the recently described particle agglutination assays were compared with transfusion results [2]. Whereas 96% of transfusions of crossmatch-positive platelets were unsuccessful, 89% of crossmatch-negative platelets produced a satisfactory increment. The crossmatch results compare favourably with those

from transfusions with HLA-selected platelets. The results in two of the centres which contributed to this Forum show that, if no HLA-matched platelets can be found, it is possible to select platelets which give a satisfactory increment by crossmatch, even for highly immunized recipients.

In only one of the centres have acid-treated platelets been used for transfusion with variable results [see Brand et al.].

In conclusion, from the information provided by our experts and from the literature, it is currently good practice to transfuse HLA-matched platelets, when HLA alloimmunization is the most likely cause of refractoriness in patients. Per centre, a minimum of approximately 2,000 donors, typed for HLA-A and -B locus, is desirable. If no HLA-matched platelets are available, platelets selected by crossmatch seem to be a good alternative.

The general importance of HPA-matched platelets cannot yet be evaluated, although in cases in which HPA antibodies with a defined specificity are present, such platelets seem to be indicated. The possible danger of post-transfusion purpura, is another reason for providing HPA-matched platelets for patients with HPA antibodies.

Prof. C.P. Engelfriet
Central Blood Laboratories
Netherlands Red Cross
Blood Transfusion Service
Plesmanlaan 125
NL-1066 CX Amsterdam (The Netherlands)

Dr. Henk W. Reesink
Medical Director
Rode Kruis Bloedbank
Amsterdam em Omstreken
Postbus 9137
NL-1006 AC (The Netherlands)

References

- 1 Ramos RR, Curtis BR, Chaplin H: A latex particle assay for platelet-associated IgG. *Transfusion* 1992;32:235-238.
- 2 Ogden PM, Asfour A, Kohler C et al: Platelet crossmatches of single donor platelet concentrates using a latex agglutination assay. *Transfusion* 1993;33:644-650.
- 3 Sugawara S, Abo T, Kumagal K: A simple method to eliminate the antigenicity of surface class I MHC molecules from the membrane of viable cells by acid treatment at pH 3. *J Immunol Methods* 1987;100:83-90.
- 4 Friedberg RC, Donnelly SF, Mintz PD: Independent roles of platelet crossmatching and HLA in the selection of platelets for alloimmunized patients. *Transfusion* 1994;34:215-220.

R. H. Aster

I
(1) We usually look for antibodies reactive with class I HLA alloantigens in a lymphocytotoxic miniscreen. If platelets that are HLA-compatible (based on typing of the patient's lymphocytes) fail to produce satisfactory posttransfusion increments, we evaluate the serum further to identify platelet-specific alloantibodies. Platelet-specific antibodies are detected in about 10% of alloimmunized patients who fail to respond to HLA-matched platelets. Antibodies against HPA-1b (P1A2), HPA-5b (Br^a) and HPA-3a (Bak^a) are encountered most often. We try to provide platelets matched for these antigens in such cases.

(2) Our current approach is to type the patient for HLA and call in donors compatible with the patient for class I HLA alloantigens. We obtain satisfactory posttransfusion increments (>7,000/ μ l/ 10^{11} platelets transfused) in about 70% of patients who receive HLA-matched platelets.

(3) The patient is typed for class I HLA antigens and the most compatible typed donor available is called. Whenever possible, we try to obtain an exact four antigen (A, B) match. When necessary, we ignore antigen splits and match within crossreactive groups [1, 2].

(4) Yes. About 5,000 donors typed for class I HLA are potentially available.

(5) Several hundred donors have been typed, at least partially, for platelet-specific alloantigens. When indicated, we try to recruit donors compatible for antigens of the HPA-1, 3, and 5 systems.

II

(1) We do not routinely do platelet cross-matching, but call donors matched for class I HLA as a first option.

(2) We do not have experience in the use of latex agglutination for crossmatching. Like others [3-5] we believe flow cytometric testing is superior to other available cross-matching methods from the standpoints of specificity, sensitivity, and practicality.

We observe a good relationship between in vitro indirect immunofluorescence and posttransfusion platelet increments. However, the number of patients we have studied to date is very small.

(3) At any given time, approximately 20-30 single-donor platelet concentrates are in inventory at our Center. These are stored at 22°C with agitation. At this time, we do not

routinely crossmatch them for patients. We expect to evaluate the feasibility and effectiveness of crossmatching platelets by flow cytometry for selected patients in the future.

III

We have examined the effect of treating platelets in citrate at pH 3.0 only in the laboratory. Like others, we have found that this treatment markedly reduces (but does not eliminate) reactions with alloantibodies specific for class I HLA. Chloroquine is not necessary, and does not augment the effect of low pH. Using selected allo- and monoclonal antibodies we have found that low pH treatment affects antigenicity by stripping β_2 -microglobulin (and probably peptide) from the class I heavy chain, causing partial denaturation of the molecule in confirmation of Neumuller et al. [6]. Since there is no significant loss of class I heavy chain from the platelet membrane, the view that low pH treatment 'elutes' class I antigens from platelets is a misconception. We have not yet satisfied ourselves that platelets treated in this way are safe and effective for transfusion to alloimmunized patients. However, this subject offers exciting possibilities for future research.

R.H. Aster, MD
The Blood Center of Southeastern Wisconsin
638 North 18th Street
PO Box 2178
Milwaukee, WI 53201-2178 (USA)

References

- 1 Duquesnoy RJ, Filip DJ, Rodey GE, Aster RH: Transfusion therapy of refractory thrombocytopenic patients with platelets from donors selectively mismatched for crossreactive HLA antigens. *Am J Hematol* 1977;2:219-226.
- 2 McElligott MC, Menitove JE, Duquesnoy RJ, Aster RH: Effective HLA-Bw4-Bw6 compatibility on platelet transfusion responses of refractory thrombocytopenic patients. *Blood* 1982;97:1-975.
- 3 Friedberg RC, Donnelly FF, Mintz PD: Independent roles for platelet crossmatching and HLA in the selection of platelets for alloimmunized patients. *Transfusion* 1994;34:215-220.
- 4 Skogen B, Christiansen D, Husebekk A: Flow cytometric analysis in platelet crossmatching using a platelet suspension immunofluorescence test. *Transfusion* 1995;35:832-836.
- 5 Kohler M, Dittmann J, Legler TJ, Lynen R, Humpe A, Riggert J, Neumeyer H, Pies A, Panzer S, Mayr WR: Flow cytometric detection of platelet-reactive antibodies and application in platelet crossmatching. *Transfusion* 1996;36:250-255.

- 6 Neumuller J, Tohidast-Akrat M, Fischer M, Mayer WR: Influence of chloroquine or acid treatment of human platelets on the antigenicity of HLA and the thrombocyte-specific glycoproteins Ia/IIa, IIb and IIb/IIIa. *Vox Sang* 1993;65:223-231.

A. Brand
B. Tomson
F.H.J. Claas

Background

In the Netherlands the National Health Authorities support the maintenance of a file of HLA-typed donors who have given their consent for thrombocytopheresis. Approximately 22,000 HLA-typed donors are registered with 7 regional Blood Banks, supporting hospitals with haematological intensive care treatment. Some of these Blood Banks are connected by a network including a match programme. This enables searches for HLA-compatible donors available in other Blood Banks. This procedure facilitates the response to a request for HLA-matched platelet transfusions and the estimation of the number of available donors for alloimmunized patients. Our Blood Bank Leidsenhage has 11,000 typed donors of which approximately 7,000 have consented to platelet donation by apheresis.

Approach If an Alloimmunized Patient Needs Platelet Transfusions

Serology: In case of suspicion of immunological refractoriness, the patient's serum is screened against a panel of 21 selected HLA-typed donors in the lymphocytotoxicity test (LCT) and 10 random donors in the platelet immunofluorescence (PIF) test.

If the LCT is positive with $\geq 60\%$ panel reactivity (PRA), we do not take the PIF results (positive or negative) into account; neither are the specificities of the HLA antibodies determined.

Donor selection: Suitable donors are selected on split antigens of the HLA-A and B

loci: A match (all HLA-split antigens between donor and recipient identical), B1U (one blank or homozygous antigen in the donor, the other 3 split antigens identical) or B2U (2 blanks or homozygous antigens present in the donor, the other 2 split antigens identical). B1X and B2X matches take one or two cross (x) reactive antigens within the broad groups into account [1]. It is only for approximately 2% of the patients that less than 5 donors with A, BU or BX matches are available in our own file. For such patients acceptable HLA mismatches are determined and introduced in the selection procedure [2].

Evaluation: Our Blood Bank provides ± 200 -250 HLA-matched platelet transfusions and $\pm 5,000$ (25,000 units) multiple random donor transfusions yearly for approximately 200 patients with aplastic thrombocytopenia in the hospitals in our own region. In 1995, for 14 patients, 203 HLA-matched platelet transfusions were administered. The posttransfusion increments of only 138 of these transfusions (68%) were made available to us. In 114 out of 138 transfusions, a corrected count increment at 1 h > 7.5 and/or at 20 h > 4.5 was obtained, indicating that in 82% of cases a satisfactory transfusion result was obtained by this selection procedure. All platelet transfusions are retrospectively cross-matched against the platelet donor by PIF and LCT, the latter in case acceptable mismatches and BX matches were used as donors. Considering the 24/138 transfusion failures, 38% of these would have been rightly predicted by the PIF crossmatch. On the other hand, 8% of the successful transfusions showed positive PIF crossmatches as well. Only 1 out of the 14 patients with multi-specific HLA antibodies supported in 1995 had concomitant HPA (3a) antibodies.

Stripped Platelets

During the past 5 years, we were confronted with an Indonesian patient (HLA A2 B46 Bw6 homozygous) with 100% PRA, for whom no donors were available. This patient was repeatedly treated successfully with multiple acid eluted random platelet transfusions. Attempts in 3 other patients were unsuccessful, due to shortcomings in standardization with regard to residual HLA antigens on platelets and platelet damage during the procedure [3].

The PIF Test

When in the presence of platelet refractoriness the LCT shows 0-60% PRA, the results of the PIF are taken into account and often the specificity of the HLA antibodies is

determined. Extended screening of 32 PIF-negative, HLA antibody-negative patients refractory for random donor platelets revealed no platelet-specific antibodies in the MAIPA [4]. Therefore, only when the PIF is positive a MAIPA is performed to exclude platelet-specific antibodies. All HPA-specific antibodies, except HPA-5^b, showed a positive PIF. Anti-HPA-5^b antibodies (which may be negative in the PIF) are only relevant in the case of failure of single donor platelet transfusions such as HLA-matched transfusions. The majority of PIF-positive LCT-negative platelets do not react in the MAIPA. Occasional antibodies are found reactive with glycoproteins II^b/III^a or IV, indicating autoreactive antibodies [4]. Patients with HLA antibodies showing a panel reactivity <60% can generally be supported with an increased dosage of random donor platelet transfusions, but it can be worthwhile for these patients to select donors negative for specific antigens.

Non-HLA Antibodies

If a patient fails to respond to A- or BU-matched platelet transfusions, titers of ABO antibodies are checked (increased titers account for platelet transfusion failures of A- and/or B-incompatible HLA-matched platelets in $\pm 10\%$ of the patients) and the MAIPA is carried out, irrespective of PIF reactivity, against 10 random donors or against the platelets of the donor whose platelets failed to cause an increment. For this purpose, HPA- and HLA-typed donors are available. During the previous 5 years (excluding babies with neonatal alloimmune thrombocytopenia) HPA antibodies were found in 5 patients (specificities: anti-HPA-1^a (n = 2), anti-HPA-3^a, (n = 1), anti-HPA-5^b (n = 1), combination of anti-HPA-1^b and -5^b (n = 1)). Four of these patients had HLA antibodies with a PRA of >60%. When HPA antibodies are found and the patient requires platelet transfusions, small numbers of HPA-typed HLA homozygous donors (A1B8; A3B7) are available in our file to select for transfusion. In such cases we also try to find suitable donors amongst siblings and eventually amongst extended family members.

Conclusion

Patients refractory for random platelet transfusions with strong multispecific HLA antibodies have, in 10–20% of cases, additional ABO and/or HPA antibodies compromising the results of HLA-matched transfusions. When a large donor file is available, for the majority of patients, A and BU split-antigen-matched platelet donors are available.

When only patients with a high PRA are eligible for matched platelet transfusions, this approach yields a success rate of 82%, despite inclusion of patients with clinical factors increasing platelet consumption. By additional PIF crossmatch prior to transfusion, the correct prediction of a successful transfusion result increases to 90%, although some suitable donors are rejected because of false-positive results. We therefore prefer to individualize the inclusion of PIF and MAIPA in the match procedure.

A. Brand
Stichting Rode Kruis Bloedbank Leidsenhage
Academisch Ziekenhuis Leiden (E3-Q)
Postbus 2184
NL-2301 CD Leiden (The Netherlands)

References

- 1 Duquesnoy RJ: Donor selection in platelet transfusion therapy of allo-immunized thrombocytopenic patients; in *The Blood Platelet in Transfusion Therapy*. New York, Liss, 1978, pp 229–243.
- 2 Claas FHJ, de Waal LP, Beelen J, Reekers P, Van de Berg-Loonen P, De Gast E, d'Amaro J, Persijn GG, Zantvoort E, Van Rood JJ: Transplantation of highly sensitized patients on the basis of acceptable HLA-A and -B mismatches. *Clin Transpl* 1989;3:185–190.
- 3 Novotny VMJ, Huizinga TWJ, Van Doorn R, Briët E, Brand A: HLA-class I eluted platelets as alternative to HLA-matched platelet transfusions. *Transfusion* 1996;36:438–444.
- 4 Novotny VMJ, Van Doorn R, Bleeker PMM, Overbeeke MAM, Claas FHJ, Brand A: Platelet transfusion refractoriness: How much effort to find an immunological cause? Submitted for publication.

M. Contreras
C. Navarrete

Immunological refractoriness to platelet transfusions is a serious complication in transfusion-dependent patients, caused by alloantibodies reacting with antigens on transfused cells (ABO on RBC, HLA on WBC; HLA and HPA on platelets). Of these, HLA alloimmunization is the most clinically rele-

vant. Thus, it is essential to use the most appropriate identification techniques for these antibodies, and to establish appropriate protocols for the management of refractoriness. Of our platelet transfusion-dependent patients, only 7–8% are immunologically refractory, through many more have HLA antibodies.

If a thrombocytopenic patient does not respond to the transfusion of random donor platelets and all other nonimmune causes of refractoriness have been excluded, the policy is to provide relatively fresh ABO identical platelets from a single donor (SD). The purpose is to eliminate the role of ABO antibodies and/or the storage lesion of platelets transfused [1]. If no increments are achieved, cytotoxic and noncytotoxic HLA antibodies are investigated using the standard lymphocytotoxicity test (LCT) and an ELISA.

The LCT is carried out using an extended incubation period to enhance sensitivity [2]. Antibody specificity is determined using a selected panel of typed cells expressing the majority of the serologically described HLA specificities. The LCT is performed with and without DTT in order to distinguish clinically relevant IgG HLA alloantibodies from lymphocyte autoantibodies [3]. Although a few IgM antibodies have been described, most immunologically refractory patients have HLA IgG antibodies. By using the extended incubation period LCT plus DTT, we detect HLA-specific antibodies in 42% of refractory patients (though not comparable, published figures range between 30 and 80 with a mean of 50%; however, these studies have not excluded autoantibodies and have been performed using the short incubation NIH cytotoxicity assay). The discrimination between allo- and autoantibodies is crucial since the majority of alloimmunized patients requiring HLA-matched platelet transfusion are bone marrow transplant recipients, with a dysfunctional immune system, who not uncommonly develop autoantibodies. Moreover, in these patients, CMV infection is associated with the occurrence of autoantibodies [4].

Noncytotoxic HLA-specific IgG antibodies are detected by an ELISA, which relies on the binding of specific HLA antibodies to wells coated with pools of solubilized HLA antigens. The assay distinguishes HLA from non-HLA antibodies [5]. ELISA achieves a 7% increase in sensitivity (42% by LCT vs. 49% by ELISA, local data).

If a patient is refractory to random platelet transfusions on at least two occasions, and HLA antibodies are detected, we provide HLA-matched platelets. Platelet-specific an-

tibodies are investigated, using the MAIPA technique, only on those patients who fail to achieve adequate increments after transfusion of HLA-matched platelets.

Our service has a panel of over 7,000 HLA-typed apheresis donors, of whom 1,100 are active and donate platelets regularly every 2–3 weeks. This allows the selection of donors in advance. Approximately 3% of these donors are homozygous for HLA-A and -B; by using them, we are able to increase the number of HLA-compatible platelets provided.

The first option for an HLA alloimmunized patient is to provide HLA A grade-compatible platelets, i.e. serologically compatible for the four HLA-A and B locus antigens at the specificity level, including serological splits. If no A matches are available, then B matches are used, i.e. serologically incompatible for crossreactive antigens, e.g. HLA-B7 vs. HLA-B27. If no A or B matches are available, a C match is chosen, i.e. serologically incompatible for a noncrossreactive antigen, e.g. HLA-B7 and HLA-B51. The number of incompatible antigens is identified by the number 1, 2, 3 or 4. For instance, B2 refers to incompatibility for 2 crossreactive antigens, C1 to incompatibility for one noncrossreactive antigen, etc. The maximum level of acceptable incompatibilities is B2 and C1.

If no A, B or C matches are available and the specificity of the HLA antibodies is known, platelets negative for the relevant antigens from a SD are selected. If this approach fails, then all available apheresis platelets are crossmatched with the patient's serum by a commercially available solid phase technique and the best match is selected for transfusion. For patients with multispecific HLA antibodies, only grade A matches are provided. If no A matches are available and/or for patients with rare HLA haplotypes, crossmatch-negative platelets are selected from our panel of apheresis donors. During 1995/96 we provided over 1,500 units of HLA-matched platelets for 62 patients (mean of 25 units/patient). Of these, 21% were A matches, 19% B, 20% C, 27% SD, and 4% were provided on the basis of a negative crossmatch. Approximately 80–90% of immunologically refractory patients supplied by our service have satisfactory responses to the transfusion of HLA-matched platelets, as judged by the 1-hour posttransfusion increment.

Preference is given to HLA-matched over crossmatched platelets because of the need to avoid HLA alloimmunization via indirect allorecognition, a complication of significance in women of childbearing age and younger patients.

Patients who fail to achieve good increments in spite of transfusion of HLA-matched platelets are investigated for the presence of HPA antibodies using MAIPA. If HPA antibodies are identified, HLA-matched and HPA-matched platelets are provided, whenever possible. Approximately 2.5% of donors in our HLA-typed panel have also been HPA typed. The majority have been HPA-1 typed, but HPA-2, -3, and -5 typed donors are also available.

We do not have data for the percentage of refractory patients with HLA antibodies who also have HPA antibodies.

Marcela Contreras, MD, FRCPath, FRCPEdin
Cristina Navarrete, PhD, MRCPath
National Blood Service –
London & the South East
North London Blood Transfusion Centre
Colindale Avenue
London NW9 5BG (UK)

.....
J. Jørgensen

Each year we transfuse about 6,000 platelet concentrate pools, each produced from 4 units of blood according to the method of Eriksson and Högman [1]. The pools are mostly given to patients undergoing autologous bone marrow transplantation or treatment of leukaemia. With the aim of avoiding alloimmunization, we normally give them platelets as a leucodepleted product. Therefore, we only see a few patients per year, who are refractory to platelet transfusions.

In the case of a refractory patient, we try primarily to find HLA-compatible platelets, and if this is not possible, we try to find a donor with acceptable antigens by a flow cytometry crossmatch.

After having tried to determine the specificity of the antibody, we select a compatible donor amongst our 15,000 HLA class I typed donors. If the antibody has a very broad panel reactivity, which is the most common situation, we select an HLA class I phenotypically compatible donor. Having found such a donor this donor undergoes plateletpheresis. The platelets obtained by apheresis are then given without a crossmatch. We have an analogous procedure if the antibody is platelet-specific. In determining the HLA class I type we take the splits into account, as far as possible.

Most of our donors continue to be donors for many years. Therefore, it is a good investment for us to type as many as possible for red cell, HLA and platelet antigens. Our donors are normally willing to donate by apheresis. A lot of the HLA-typed donors have also accepted to be unrelated bone marrow donors.

Concerning the result of this approach, unfortunately we do not have yet enough exact data. These patients are very rare. However, the clinical data normally show that the posttransfusion platelet counts are sufficiently high and that the bleedings have stopped. Our good results are achieved because the big pool of HLA-typed donors makes it possible to find a donor with a perfect match.

We detect HLA antibodies in approximately 20 refractory patients per year, and only 1–2 times per year are we unable to find a compatible donor and have to find a suitable donor by a crossmatch. We find platelet-specific antibodies only a few times per year. If there are HLA antibodies but no suitable donor, we try to find the most suitable donors in our file and then do a crossmatch by flow cytometry [2]. We do not freeze samples from our donors but use freshly taken samples

.....
References

- 1 Norol F, Kuentz M, Cordonnier C, Beaujean F, Haioun C, Vernant JP, Duedari N: Influence of clinical status on the efficiency of stored platelet transfusion. *Br J Haematol* 1994;86:125–129.
- 2 Garner SF, Petrochilos J, Brown CJ, Cavanna S, Chamarin I, Navarrete C: Clinically significant anti-HLA A2. *Immunohaematology*. In press.
- 3 McCloskey DJ, Brown J, Navarrete C: Serological typing of HLA-A, -B and -C antigens; in Hui KM, Bidwell JL (eds): *Handbook of HLA Typing Techniques*. London, CRC Press, 1993, pp 175–248.
- 4 Hebart H, Einsele H, Klein R, Fischer I, Buhler S, Dietz K, Jahn G, Berg PA, Kanz L, Muller CA: CMV infection after allogeneic bone marrow transplantation. *Br J Haematol* 1996;95:138–144.
- 5 Kao U, Scornik JC, Small SJ et al: Enzyme-linked immunoassay for anti-HLA antibodies: An alternative to panel studies by lymphocytotoxicity. *Transplantation* 1993;55:192–196.

from the primarily chosen donors. It is so easy to get in contact with our donors, that it is not worthwhile storing samples.

Concerning acid-treated platelets, we have no experience using them for treatment, but are only using the technique for antibody characterization in the laboratory [3]. For the time being, our last choice is just to give random presumably incompatible platelets, but this will often stop the bleeding without significant harm to the patient.

J. Jørgensen, MD, DSc
Regional Blood Transfusion Center
Aarhus University Hospital
Skejby Sygehus
Brendstrupgaardsvej
DK-8200 Aarhus N (Denmark)

References

- 1 Eriksson L, Högman CF: Platelet concentrates in an additive solution prepared from pooled buffy coats. *Vox Sang* 1990;59:140–145.
- 2 Skogen B, Christiansen D, Husebekk A: Flow cytometric analysis in platelet crossmatching using a platelet suspension immunofluorescence test. *Transfusion* 1995;35:832–836.
- 3 Kurata Y et al: Acid treatment of platelets as a simple procedure for distinguishing platelet-specific antibodies from anti-HLA antibodies: Comparison with chloroquine treatment. *Vox Sang* 1990;59:106–111.

M. F. Murphy
R. Curtis
A. H. Waters

For the management of patients requiring repeated platelet transfusions, we follow the recommendations of the British Committee for Standards in Haematology [1], as follows: (1) type patients for HLA-A and -B antigens at an early stage; (2) use random donor platelet concentrates for initial platelet support; (3) if refractoriness occurs, determine whether clinical factors, which may be associated with nonimmune consumption of platelets [2], are present and test the patient's serum for HLA

antibodies; (4) if HLA alloimmunization is the most likely cause of refractoriness, use HLA-matched platelet transfusions; (5) if there is no improvement with HLA-matched transfusions, platelet crossmatching (i.e. testing the patient's serum against the lymphocytes and platelets of one or more of the HLA-matched donors) may identify the cause of the problem and help with the selection of compatible donors.

The early identification of refractory patients who are alloimmunized is helpful in avoiding the waste of transfusing HLA-matched platelets to patients with nonimmune refractoriness. This is facilitated by testing the serum of patients receiving repeated platelet transfusions for HLA antibodies prior to intensive transfusion support and at regular intervals, e.g. weekly.

We have access to a panel of HLA-typed platelet donors through the National Blood Service. The best-matched HLA-compatible donors are selected according to the usual criteria including crossreactive HLA antigens, and platelets are collected by apheresis either in the hospital apheresis unit or in one of our local Transfusion Centres. An attempt is made to determine the specificity of any HLA antibodies detected, although this is not possible in many multitransfused patients. Identification of the specificity may assist in the selection of compatible donors when fully matched donors are not available.

We test for platelet-specific antibodies in two circumstances: (1) in patients who appear to have immune refractoriness in whom no HLA antibodies are detectable, and (2) to identify the cause of poor responses to HLA-matched platelet transfusions. We use the lymphocytotoxicity test, lymphocyte and platelet immunofluorescence tests and the MAIPA technique to crossmatch the patient's serum with donor platelets and lymphocytes. However, platelet-specific antibodies are an uncommon cause of platelet refractoriness in our experience [3].

We have reviewed the use of HLA-matched platelet transfusions over the last year (total 49). No posttransfusion platelet count was performed after 14 transfusions, usually because the patient was being discharged or was attending as an outpatient. The remaining 35 HLA-matched transfusions were given to 10 patients, and only 8/35 (23%) produced a satisfactory platelet recovery (>20% of the transfused platelets circulating at 20 h posttransfusion [4]). We found a clear relationship between HLA match grade and response; 50% of A grade matches had a good response compared with 15% of other

match grades. Platelet-specific antibodies were not detected in the patients having poor responses to HLA-matched transfusions, and the poor responses to HLA-matched transfusions appeared to be due to either HLA incompatibility or nonimmune platelet consumption associated with septicemia and its treatment with antibiotics and antifungal drugs.

The response rate of 50% for even grade A HLA-matched platelet transfusions in our study was slightly lower than the generally accepted response rate of around 70% to well-matched donors [5]. However, in our small study, patients were not selected to be without factors known to be associated with nonimmune platelet consumption at the time of the transfusion, but it can be argued that this reflects the situation in routine clinical practice. The 15% success with poorer degrees of HLA match suggests that we should aim to provide the best possible HLA match, and that perhaps we should be attempting to find better methods for providing HLA-matched platelets.

Consideration could be given to using platelet crossmatching of random donors in routine donor selection for refractory alloimmunized patients [6, 7]. This would avoid the need for maintaining costly HLA-typed platelet panels, but this approach would not be successful in patients with multispecific HLA antibodies reacting with 100% of the panel.

Another approach to the management of refractory alloimmunized patients is the removal of HLA antigens from platelets prior to transfusion. Chloroquine and acid treatment are established techniques in platelet serology for the differentiation of HLA and platelet-specific antibodies, and platelets 'stripped' of HLA antigens may improve responses in alloimmunized patients. Using this technique, we found that it is difficult to remove HLA antigens effectively while maintaining platelet function [8]. However, others have shown that this may be worthy of further investigation [9].

Acknowledgement

We thank Dr. Cristina Navarrete, National Blood Service, London & South East Zone, for information about the grade of HLA matching of the platelet donors.

M. F. Murphy
R. Curtis
A. H. Waters
Department of Haematology
St. Bartholomew's Hospital
West Smithfield
London EC1A 7BE (UK)

References

- 1 British Committee for Standards in Haematology: Guidelines for platelet transfusions. *Transfus Med* 1992;2:311–318.
- 2 Doughty HA, Murphy MF, Metcalfe P, Rohatiner AZS, Lister TA, Waters AH: Relative importance of immune and non-immune causes of platelet refractoriness. *Vox Sang* 1994;66:200–205.
- 3 Murphy MF, Waters AH: Platelet transfusions: The problem of refractoriness. *Blood Rev* 1990; 4:16–24.
- 4 Waters AH, Minchinton RM, Bell R, Ford JM, Lister TA: A cross-matching procedure for the selection of donors for alloimmunized patients. *Br J Haematol* 1981;48:59–68.
- 5 Slichter SJ: Mechanisms and management of platelet refractoriness; in Nance SJ (ed): *Transfusion Medicine in the 1990s*. Arlington, American Association of Blood Banks, 1990, pp 95–179.
- 6 Rachel JM, Summers TC, Sinor LT, Plapp FV: Use of a solid phase red blood cell adherence method for pretransfusion platelet compatibility testing. *Am J Clin Pathol* 1988;90:63–68.
- 7 Rule SA, Erber WN, Lown JA, Dale BJ, Herrmann RP, Davies JM: Cross-match platelets in bone marrow transplantation. *Pathology* 1994; 26:288–290.
- 8 Murphy MF, Metcalfe P, Caples R, Waters AH: Function of acid-treated platelets for transfusion. *Br J Haematol* 1992;82:176–178.
- 9 Novotny VMJ, Huizinga TWJ, van Doorn R, Briet A, Brand A: HLA class I-eluted platelets as an alternative to HLA-matched platelets. *Transfusion* 1996;36:438–444.

Simon Panzer

Martin Kurz

Paul Höcker

Wolfgang Richard Mayr

Our institution provides only single donor apheresis platelets, which are processed and leukocyte-reduced at the Department of Transfusion Medicine. More than 90% of all platelet concentrates are produced in this way for adult and childhood patients with haemato-oncological disease, including those undergoing haematopoietic stem cell transplantation. In many patients refractoriness to platelet transfusions results from the patients' clinical condition, and is not due to platelet antibodies [1]. The attending clinician defines a patient as 'refractory' if two consec-

utive transfusions within 2 days are not followed by a satisfactory platelet increment (12 h after transfusion corrected count increment, CCI, $>5,000 \times 10^9/\text{litre}$), irrespective of any clinical condition which is known to adversely affect the posttransfusion increment.

In these patients we determine antibodies against HLA class I that are reactive with platelets, and HPA antibodies. Sera are screened for antibodies by the MAIPA technique [2] using a panel of selected blood group O platelets homozygous for HPA-1, -2, -3 and -5a or b specificities. A platelet pool from 60 donors is used for the determination of HLA antibodies. Thereby, by the MAIPA technique 90% of all HLA class I antigens are detectable with any antisera after immunoprecipitation with anti- β_2 -microglobulin. The HLA antibody specificity (by the lymphocytotoxicity test) is only determined if the patients' HLA class I phenotype is unknown. Otherwise, donors are selected by HLA matching [3, 4], without considering splits, from a donor pool of more than 2,500 HLA-typed individuals. No further crossmatch is performed once a donor has been selected. The same approach is used if an HPA-specific antibody is identified. The donor selection is made from 400 blood group O donors, typed for HPA-1, -2, -3 and -5, in addition to HLA. This approach allows to achieve a satisfactory platelet increment (CCI $>5,000 \times 10^9/\text{litre}$, 12 h after transfusion) in 60% of refractory patients.

All patients scheduled for haematopoietic stem cell transplantation are screened for platelet-reactive antibodies, and a crossmatch by the MAIPA technique is performed against the prospective marrow donor. Patients undergoing allogeneic bone marrow transplantation, who become refractory to random donor platelets, will preferably receive platelets from the marrow donor or an HLA-identical sibling. With this approach we achieve in these refractory patients a satisfactory platelet increment (CCI $>5,000 \times 10^9/\text{litre}$) in 90% of transfusions.

Platelet-specific antibodies are detected in 46% of refractory patients. Thirty percent of these antibodies are panreactive, and 20% are directed against HPA specificities [5]. Of note, antibodies are transient in some patients, or titres wax and wane [5, 6]. Acid-treated platelets have not been used in our institution for transfusion.

Simon Panzer, MD
Clinic for Blood Group Serology and
Transfusion Medicine
University Vienna
Währinger Gürtel 18–20
A–1090 Vienna (Austria)

References

- 1 Panzer S, Maier F, Höcker P, Mayr WR, Hinterberger W: Thrombozytentransfusion: Plättchenanstieg in abhängigkeit von klinischen und immunologischen Voraussetzungen. *Infusionstherapie* 1987;14:10–14.
- 2 Kiefel V, Santoso S, Weisheit M, Mueller-Eckhardt C: Monoclonal antibody-specific immobilization of platelet antigens (MAIPA): A new tool for the identification of platelet-reactive antibodies. *Blood* 1987;70:1722–1726.
- 3 Yankee RA, Graff KS, Dowling R, Henderson ES: Selection of unrelated compatible platelet donors by lymphocyte HL-A matching. *N Engl J Med* 1973;288:760–764.
- 4 Wu KK, Hoak JC, Koepke JA, Thoson JS: Selection of compatible platelet donors: A prospective evaluation of three cross-matching techniques. *Transfusion* 1977;17:638–643.
- 5 Kurz M, Greinix H, Knöbl P, Kalhs P, Pober M, Höcker P, Mayr WR, Panzer S: Specificities of anti-platelet antibodies in multitransfused patients with haemato-oncological disorders. *Br J Haematol* 1996;95:564–569.
- 6 McGrath K, Wolf M, Bishop J, Veale M, Ayberk H, Szer J, Cooper I, Whiteside M: Transient platelet and HLA antibody formation in multitransfused patients with malignancy. *Br J Haematol* 1988;68:345–350.

Charles A. Schiffer

Although prolonged, severe thrombocytopenia is a routine consequence of therapy for patients with hematological malignancies, clinically significant or fatal hemorrhage is an unusual occurrence, in part because of the ready availability of platelet transfusions of good quality. When serious hemorrhage does occur, it is most commonly in association with coagulopathies (i.e., patients with acute promyelocytic leukemia) or in patients refractory to platelet transfusions because of alloimmunization. There are many clinical conditions associated with poor platelet count increments following transfusion including splenomegaly (sometimes underappreciated on physical examination), fever, infection, subclinical disseminated intravascular coagulation, occasionally issues related to platelet storage, possibly the administration of certain medications such as amphotericin B and other recipient factors which are poorly understood [1]. Indeed, the mechanisms by which many of these clinical

factors may compromise posttransfusion increments have not been well evaluated and their effects may be somewhat exaggerated in many texts because excellent increments are often obtained in the sickest of patients in whom many of these clinical factors are present. Alloimmunization probably accounts for a minority of refractory episodes and it has been our impression in the last decade, even before the advent of leukocyte filtration techniques, that the incidence of alloimmunization in patients with leukemia had been decreasing [2].

While many of these clinical factors compromise increments, they usually do not absolutely eliminate posttransfusion increments as is common in alloimmunized patients. Another clinical clue to the diagnosis of alloimmunization is the development of recurrent transfusion reactions. However, this can be misleading because it has recently become clear that the majority of such reactions are a consequence of infusion of cytokines released during platelet storage [3]. Hence, because of the difficulties and expense associated with the identification of histocompatible donors, we recommend and perform platelet antibody testing either using standard lymphocytotoxicity or platelet antibody screening techniques to confirm the presence of alloantibodies in patients refractory to platelet transfusions [4, 5]. Both techniques are reliable, although anecdotally, we have sometimes noted that the rapid solid-phase platelet antibody test (Capture P, IMMUCOR, Norcross, Ga., USA) can be positive before the antibody can be detected by lymphocytotoxicity, perhaps because one is a simple binding assay and the other requires a more complex series of complement fixing reactions.

There are a large number of studies available documenting the utility and limitations of donor selection by HLA typing for alloimmunized patients [6]. Some of the range in the reported results is certainly a consequence of the heterogeneity of antibody specificity and strength in different alloimmunized patients. Some patients with highly reactive antibody screens are extraordinarily refractory to virtually all transfusions, while others, often with less reactive antibody screens, can be supported with partially mismatched transfusions [7]. Although most reports would suggest that antibodies against platelet-specific antigens are uncommon, their presence can also obviously affect the interpretation of the results of HLA-matched transfusions. In addition, transfusions to clinically stable, non-alloimmunized patients are occasionally not successful for poorly understood reasons.

Thus, all of these factors come into play and account for the variable success seen with HLA-matched transfusions.

Nonetheless, it is clear that donor selection by HLA matching can be effective in the majority of patients [6, 7]. It offers the advantage of a well-characterized, computerized donor pool which, in large centers, can generally be accessed reasonably rapidly as alloimmunized patients are identified. In recent years, partially because of the development of rapid and relatively simple platelet antibody testing, donor selection by this technique has also garnered increased attention. Our approach has been to initially attempt to identify donors by HLA selection. If sufficient numbers of donors are not available or initial experience with such transfusions is unsuccessful, we have utilized donor selection by platelet crossmatching. Our transfusion service utilizes pooled random donor platelets for the majority of our recipients and we have identified a technique in which samples of individual units of platelet concentrates are cross-matched, with small aliquots of compatible units transfused individually [5]. When these transfusions of 1–3 units produce an acceptable corrected count increment, we attempt to identify the original blood donors of these units and ask them to serve as apheresis donors. This approach has the logic of the donor having 'passed' both an *in vitro* and *in vivo* 'crossmatch'. In addition, the increments achieved with the smaller transfusions can often be clinically meaningful to severely alloimmunized patients. For such heavily immunized patients, it is often necessary to cross-match more than 100 units to find 1 or 2 donors compatible *in vitro*. When such units are transfused, they have produced 'successful' corrected count increments more than 80% of the time.

Of interest is that sometimes the HLA types of these donors are quite discrepant from that of the recipients (all of whom had broadly reactive lymphocytotoxic antibody) and these donors would not have been identified by computer searches of HLA types. It has however, then been possible to utilize a second 'search strategy' based on the successful donors' HLA type to identify subsequent donors. This approach has also been applicable in more emergency situations when it has been impossible to immediately identify an HLA-matched donor either from our own resources or the resources of the community blood center. The solid-phase antiplatelet assay can be performed within 1–2 h and can rapidly identify potentially compatible platelets in emergency situations. It is also suitable

for use in centres where there are large numbers of apheresis products produced daily, although the observation that it is sometimes necessary to screen hundreds of units makes this less practical for some recipients.

In summary, our center utilizes a combination of donor selection by HLA typing and platelet antibody crossmatching. We have not performed comparative trials of the utility or costs associated with each technique (or the comparative sensitivity of other platelet antibody tests) but have clearly noted circumstances in which they are both complementary to each other and combinations of the techniques are often utilized in individual patients. This may be particularly true in patients with less common HLA phenotypes in whom the number of HLA-matched donors is limited.

Charles A. Schiffer, MD
Professor of Medicine and Oncology
Marlene and Stewart Greenebaum Cancer
Center (formally University of Maryland
Cancer Center)
University of Maryland School of Medicine
22 S. Greene Street
Baltimore, MD 21201 (USA)

References

- 1 Bishop JF, McGrath K, Wolf MM, Matthews JP, De Luise T, Holdsworth R, Yuen K, Veale M, Whiteside MG, Cooper IA, Szer J: Clinical factors influencing the efficacy of pooled platelet transfusions. *Blood* 1988;71:383.
- 2 Lee EJ, Schiffer CA: Serial measurement of lymphocytotoxic antibody and response to non-matched platelet transfusions in alloimmunized patients. *Blood* 1987;70:1727–1729.
- 3 Heddle JM, Klama L, Singer J, Richards C, Fedak P, Walker I, Kelton JG: The role of plasma from platelet concentrates in transfusion reactions. *N Engl J Med* 1994;331:625–628.
- 4 Hogge DE, Dutcher JP, Aisner J, Schiffer CA: Lymphocytotoxic antibody is a predictor of response to random donor platelet transfusion. *Am J Hematol* 1983;14:363–370.
- 5 O'Connell BA, Lee EJ, Rothko K, Hussein MA, Schiffer CA: Selection of histocompatible apheresis platelet donors by crossmatching random donor platelet concentrates. *Blood* 1992;79:527–531.
- 6 Duquesnoy RJ, Filip DJ, Rodey GE, Rimm AA, Aster RH: Successful transfusion of platelets mismatched for HLA antigens to alloimmunized thrombocytopenic patients. *Am J Hematol* 1977;2:219.
- 7 Hussein MA, Lee EJ, Fletcher R, Schiffer CA: The effect of lymphocytotoxic antibody reactivity on the results of single antigen mismatched platelet transfusions to alloimmunized patients. *Blood* 1996;87:3959–3962.